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Light-Chain Paraproteins With Lupus Anticoagulant Activity

Zahida Yasin,[1,5]* Donald Quick,[2] Perumal Thiagarajan,[3] Denise Spoor,[4] Juan Caraveo,[1] and Joseph Palascak[5]

[1]Department of Internal Medicine, Division of Hematology/Oncology, Scott & White Clinic and Memorial Hospital, Scott, Sherwood and Brindley Foundation, Texas A&M University Health Science Center, College of Medicine, Temple, Texas
[2]Joe Arrington Cancer Research and Treatment Center, Lubbock, Texas
[3]Department of Internal Medicine, Division of Hematology, University of Texas Medical Center, Houston, Texas
[4]Department of Pathology, Scott & White Clinic and Memorial Hospital, Scott, Sherwood and Brindley Foundation, Texas A&M University Health Science Center, College of Medicine, Temple, Texas
[5]Department of Internal Medicine, Division of Hematology/Oncology, University of Cincinnati College of Medicine, Cincinnati, Ohio

A patient with newly diagnosed multiple myeloma manifested by urine kappa light-chain excretion and a small monoclonal spike (0.4 g/dl), presented with lower extremity deep venous thrombosis. A preheparin plasma-activated partial thromboplastin time (aPTT) was prolonged at 68 sec (normal control 26-42 sec). Additional studies confirmed the presence of lupus anticoagulant activity in the serum: the modified Russell Viper Venom Time (MRVVT) was 73 sec (normal control 24–42 sec) and with a 50:50 mix of the patient's plasma and pooled normal plasma, the MRVVT remained prolonged. Kappa light chains (LC) were isolated from the patient's urine and their purity confirmed by electrophoresis and immunofixation using specific immunoglobulin antisera. The patient's LC mixed with pooled normal plasma demonstrated LA activity by in vitro clotting tests (plasma-activated partial thromboplastin time 62 sec, with normal control of 45 sec), MRVVT of 44 sec with normal control of 35 sec. Purified urinary kappa light chains from a control patient with multiple myeloma and normal clotting studies, failed to prolong either the plasma-activated partial thromboplastin time or the MRVVT. We hypothesize that kappa LC in our patient demonstrated LA activity, which was unique to these LCs. Paraproteins with LA activity, to date, have included only intact immunoglobulins (Ig). While intact Ig paraproteins have been reported to possess LA activity, this is the first report to our knowledge of light-chain paraproteins possessing similar activity and resulting in clinically evident thrombosis. Light chain paraproteins could serve as useful models for further study of the mechanisms of activity of acquired LA inhibitors. Am. J. Hematol. 62:99–102, 1999. © 1999 Wiley-Liss, Inc.

Key words: lupus anticoagulant; phospholipid; free light chain; multiple myeloma

INTRODUCTION

Lupus anticoagulants (LAs) are immunoglobulins that interfere with phospholipid-dependent in vitro coagulation tests [1–3]. The epitope for most of these antibodies consists of a complex of anionic phospholipid and plasma proteins such as β2 Glycoprotein-1 (B2G1P1) and prothrombin [1–3]. Monoclonal immunoglobulins in plasma cell neoplasms have been shown to have antigen binding activity, which may lead to some of the clinical manifestations [4]. These proteins have also been shown to have immunological specificity towards anionic phospholipid and behave as a LA [5]. We recently had a patient with deep vein thrombosis and light-chain (LC) myeloma, who also had a LA. We isolated monoclonal LC from the urine and showed that free LCs did behave as a LA in this patient.

Clinical Summary

A 77-year-old woman who presented with severe low-back pain and unilateral lower extremity swelling was

*Correspondence to: Dr. Zahida Yasin, Division of Hematology-Oncology, University of Cincinnati, College of Medicine, K Pavilion ML 0508, 231 Bethesda Avenue, Cincinnati, Ohio 45267-0508.

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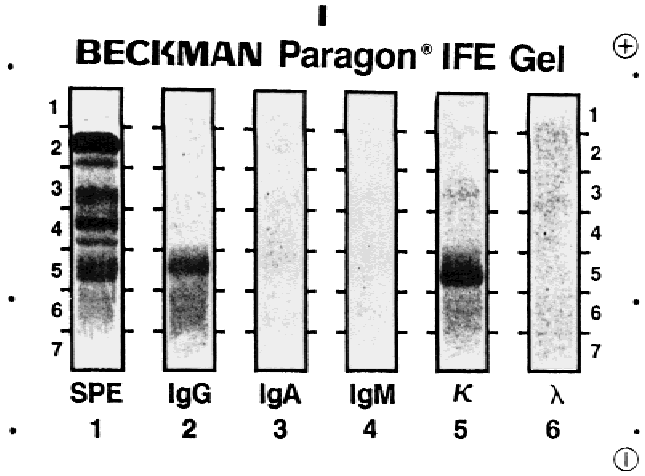

Fig. 1. Immunofixation of patient serum at time of diagnosis showing free kappa LCs.

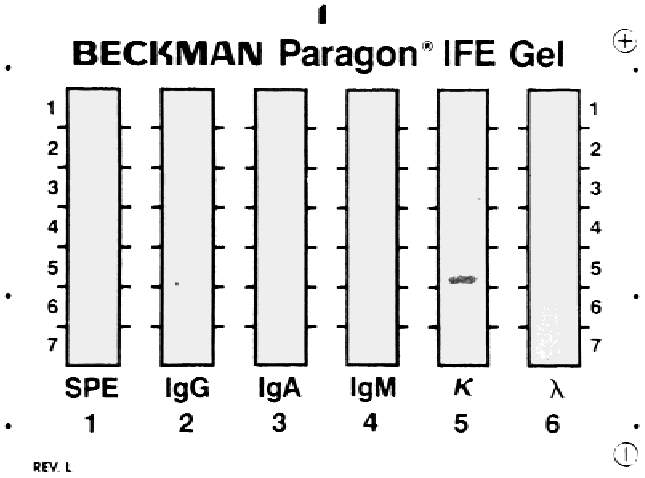

Fig. 2. Immunofixation of patient's urine after separation of kappa LCs by ammonium sulfate precipitation.

found to have acute deep vein thrombosis on Doppler ultrasonography. Further work-up with a skeletal survey showed lytic bony lesions, involving the spine and proximal femur. A bone marrow biopsy showed the presence of atypical plasma cells constituting 40% bone marrow cellularity. Further laboratory evaluation revealed panhypogammaglobulinemia and a small (0.4 g/dl) monoclonal spike in the region of the gamma-globulin fraction (Fig. 1). Quantitation for protein in a 24-hr specimen of urine showed 10 g of kappa LCs. Initial coagulation screening of the patient's plasma showed an elevated partial thromboplastin time (aPTT). Further testing confirmed the presence of a circulating LA inhibitor in the patient's plasma. Purified LCs from the patient's urine (Fig. 2) showed LA inhibitor activity (Tables I and II).

TABLE I. Prolonged aPTT With LA LC Compared to Control LCs Both Mixed With Pooled Normal Plasma

Patient's urine LCs (75 mg/dl) + PNP	aPTT (Dade's FSH aPPT reagent) control 45-46 sec
Sample A (LC, LA)	62
Sample B (LC-Control)	46

TABLE II. Modified/Dilute Russell's Viper Venom Time

	Modified RVVT (PNP + reagent grade H_2O) (control = 34 sec)
Urine	
Sample A	44
Patient's urine LCs (75 mg/dl) + PNP	
Urine	
Sample B	34
Negative control LCs (75 mg/dl) + PNP	
Plasma	Control 24-40
Patient's plasma sample	73

LABORATORY METHODS

Standard clinical laboratory methods were used for prothrombin time, PTT, serum electrophoresis, immunofixation, and anticardiolipin antibody.

Purification of LCs

Ammonium sulfate precipitation technique [6] was used to separate the LCs from 24-hr urine specimens of two myeloma patients with Bence Jones proteinuria (kappa light chains). Sample A (Fig. 3) was prepared from LCs isolated from the patient with coagulopathy. Control kappa LCs isolated from the urine of another patient with LC myeloma whose coagulation tests were normal, were used to prepare test sample B (Tables I and II; Fig. 3). In addition, LCs were further purified by gel filtration on a column (2 × 50 cm) of Sephadex G 25 (Pharmacia Biotechnology Inc, Piscataway, NJ). The purity of the isolated LCs was confirmed by electrophoresis and immunofixation using type-specific anti-sera.

LA Activity of Isolated LCs

The effect of LCs on aPTT was determined in a modified aPTT assay, consisting of 100 ml of normal plasma, 90 ml of phospholipid reagent, 10 ml 10 mM ellagic acid and 100 ml of buffer containing various concentrations of LCs. After 5 min incubation, clotting was initiated by the addition of 100 ml of 30 mM $CaCl_2$. The LA effect of the isolated light chains was also tested on dRVVT [7,8]. A kaolin clotting time [9] was also indicative of LA

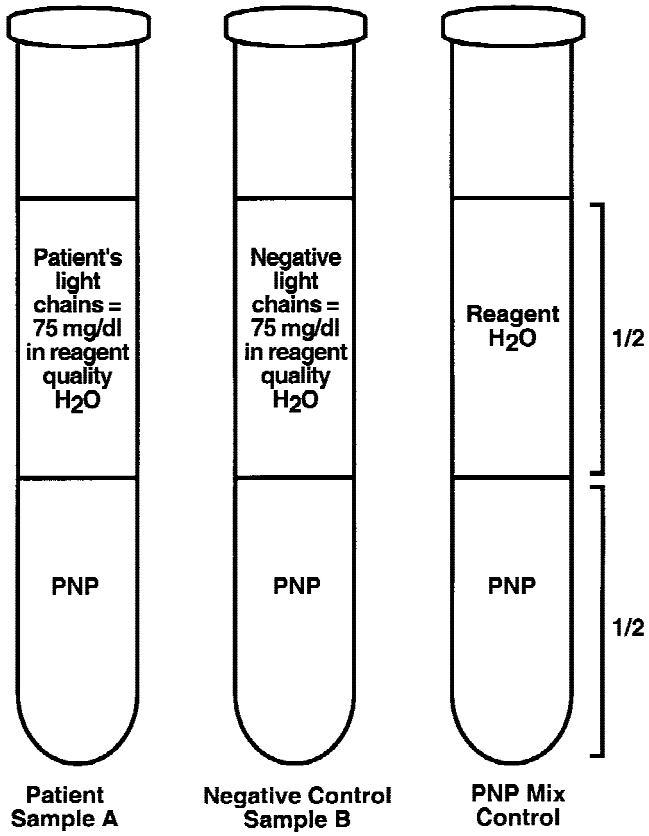

Fig. 3. Patient sample A = patient's purified urine LCs suspended in reagent grade H_2O (75 mg/dl) + PNP (50:50 mix). Negative control sample B = purified LCs from an unrelated myeloma patient without coagulopathy suspended in reagent grade H_2O (75 mg/dl) + PNP (50:50 mix). PNP mix (normal control), pooled normal plasma in reagent grade H_2O (50:50 mix).

activity (data not shown) in sample A and not in control sample B.

RESULTS AND DISCUSSION

The initial plasma coagulation screen showed the presence of a LA in this patient with LC myeloma (Table II). Since many monoclonal immunoglobulins have been shown to interfere with the normal coagulation cascade, we tested whether the abnormal LC may be causing the coagulation abnormalities. To determine this, we isolated the free kappa LCs from the urine. Addition of purified LC to normal plasma caused a concentration-dependent prolongation of aPTT (Table III). Furthermore, this LC has the characteristics of lupus anticoagulant as seen by its effect on dRVVT and aPTT (Tables I and II). These results show that the isolated LC acts as a LA.

The immunological specificity of antigen-antibody interactions are due to the sequence diversities of the amino terminal regions of heavy and light chains, which form the antigen binding site [8]. The heavy chain is the predominant contributor to the free energy of binding and interaction with antigen in a specific manner, whereas the contribution of the LC to antigen binding has been thought to be limited [10]. Antigen binding by isolated LCs has been reported occasionally [11]. Here we show that free LCs from the patient possess LA activity. The nature of this interaction may be a specific immunological interaction through the aminoterminus as has been reported for certain LCs [11]. However, it may also represent a hydrophobic interaction with phospholipids. Hydrophobic interactions between phospholipids and monoclonal immunoglobulins have been previously documented. Furthermore, other nonimmunological interactions of LCs have also been reported [12]. Despite its LA effect, no significant phospholipid-binding activity can be demonstrated in ELISA assays with or without B2G1P1. This may be due to the conformation of the phospholipid in the aPTT reagents compared to the dried lipids in ELISA assays.

TABLE III. Dose Response Profile*

LC concentration (μg/ml)	aPTT (sec)	
	Patient	Control
250	51	34.5
125	41.5	34.1
62.5	39.9	34.7
31.5	37.5	35.2
15.6	37.8	33.9
7.8	37	34.8
3.9	37.8	35.1
1.9	35	34.8

*The effect of patient's LA LCs mixed with pooled normal plasma on aPTT in sec, compared with control LCs in increasing concentrations.

In summary, we report LA activity of monoclonal LCs in a patient with multiple myeloma. To our knowledge, this is the first report of free human LCs demonstrating LA activity. Whether this is unique to the specific LC that was being produced by the myeloma clone in our patient, or is a more generalized phenomenon related to a particular subclass of kappa LCs needs to be explored further. Even more challenging will be the substantiation of specific mechanisms by which these LCs interact to produce LA inhibitor activity and the clinically evident thrombosis. Finally, monoclonal LCs uncontaminated by plasma and its component proteins can serve as an excellent model to further study mechanisms of thrombosis associated with LA activity.

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